

Microbial Quality of Ice Creams sold by street vendors - A case study in Hyderabad

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Abstract

Ice-cream is indeed one among the widely accepted dairy products among all age groups. The Process of Ice cream making involves various stages. At any stage of manufacture there can be a chance of microbial contamination from humans and environment sources. This study aims to assess the Microbial Quality of Local made ice-creams sold by the street vendors on Push carts in the Hyderabad City. Randomly collected 20 ice creams which are local made and packed in cups in the vicinity of University College for Women, Koti were collected and studied to determine the colony forming units per gram of the sample. Bio-chemical tests like Indole test, Vogues's Proskauer test, citrate utilisation test; violet bile agar test, catalase test and methylene blue reduction test were done to identify the presence of coli forms and any other pathogenic microorganisms. The viable count of microbes was found in the range of 1×10^5 to 3×10^5 . 70 % of the samples were found to be negative for the biochemical tests indicating that the quality of ice creams from local vendors was good. 7 out of 20 samples were positive for the Indole test while 4 out of 20 showed positive results in Vogues- Proskauer test. These tests were done to study the presence of E. coli, coli forms, streptococci etc. The occurrence of microbes in 30% of the samples might be due to careless handling during the preparation, processing and packing of ice creams. Vendors of local areas like Santosh Nagar, Babanaga, are maintaining good hygienic environment for the preparation and storage of ice creams. While those manufacturing at Yakutpura, Talabkatta have some poor hygienic conditions which lead to contamination of microbes in the samples collected.

Introduction

Ice cream is a nutritionally enriched congealed dairy product consumed by all, particularly children, during summer [1]. It is a frozen product made from cream and many other ingredients apart from milk and sugar. Quality of ice cream depends on both extrinsic factors that include manufacture procedure, and intrinsic factors that include proportion of ingredients used. The ice cream market growth picked up after de-reservation of the sector in 1997. Of the total size of Rs 15-16 billion, around 30-32% is in the hands of organized sector valued at Rs 4.9 billion, rest all is with the unorganized sector [2]. Formation of ice cream involves a processed method that is preparation, handling and packaging. During this process the product may get contaminated with microbes and lead to infections to human body. Primary sources of microbial contamination to ice cream include water and raw milk whereas secondary sources include flavouring agents, utensils and handling. Although pasteurization, freezing and hardening steps in production can reduce most of the microbial hazards, but still numerous health hazards are persistent due to various conditions. An investigation conducted among vendors showed a lack of education and training; these vendors need information about food preparation and storage practices that reduce microbiological contamination of foods.

Many psychrophiles and psychro tolerant microorganisms like *Listeria monocytogens*, *Staphylococcus aureus*, *Bacillus*, *Salmonella*, *Shigella*, *Streptococcus*, *Pseudomonas*, *Campylobacter*, *Brucella* and *Coliform* bacteria are generally present in ice cream [3].

Microbiological and bio chemical tests of the ice cream samples help to know the amount of *Coliforms*, *E.coli*, *Streptococcus*, etc., present in the ice cream. A large majority of urinary tract infections are caused by *Coliform* organisms. Most *E.coli* strains are harmless but some pathogenic organisms can cause serious food poisoning in humans. *Staphylococcus aureus* is a common cause of skin infections, respiratory diseases and food poisoning. coli form bacteria are one of most important indicator organisms that are most commonly used to ensure food safety include coli form bacteria, faecal coliform bacteria, *E.coli*, total enterococcus species and aerobic plate count [4].

Access to good quality, safe and nutritious food is considered a basic right of the people. Consumption of unsafe, contaminated food leads to food-borne diseases which cause considerable morbidity and mortality. The diseases transmitted by food are commonly referred to as food poisoning and are characterized by abrupt onset of gastrointestinal disturbances viz. abdominal pain, vomiting and diarrhoea Ice cream is an excellent medium for the growth of many microorganisms some of which

cause diseases in human beings e.g. Cholera, typhoid, bacillary dysentery [5]. Historically, it has been responsible for a number of outbreaks of food-borne illness [6]. Possible source of contamination are the processing methods that are used in preparation, inappropriate holding temperature, poor personal hygiene [7, 8]. Hence, the present study was taken up to determine the bacteriological quality of Local made and sold ice-creams in Hyderabad city, and to associate the organisms with their potential risk to the public health.

Materials and methods:

Twenty Ice-cream samples randomly collected from selected street vendors in and around the University College for Women, Koti, Hyderabad during the month of March were analyzed for total viable colony count and the presence of Coliforms, *Aeromonas hydrophilia*, *E.coli*, *Klebsiella oxytoca*, *Streptococcus faecalis*, *Enterobacter* species, *Staphylococci*, *Serratia marcescens*, *Vibrio alginolyticus* etc. were determined by conducting simple staining and Biochemical tests. Bio-chemical tests included MBRT (Methylene-Blue Reduction Test), Voges-Proskauer test, citrate utilisation test, carbohydrate fermentation test, violet red bile salt agar test, catalase test. All the samples were subjected to the tests to obtain triplicate values. All the samples were collected within a radius of 5 to 7 Km and were transported in an Ice box with in a maximum transportation time of 20 minutes.

Aerobic colony counts:

Aerobic Colony counts (ACC) were performed using spread plate method [9, 10]. Nutrient agar plates (HI-MEDIA) were inoculated with 0.1 ml of 10^{-5} times diluted cup ice cream samples and incubated at 37°C. After 24 h of incubation, the total numbers of Colonies that appear were counted with the help of digital colony counter (LABFITTM, Ambala, India). The values for total viable count were compared with the standards as per the Food Safety and Standards Act, 2011 guidelines Standard for Ice Cream as per FSSA (Food Safety and Standards Act, 2011) mentioned in **APPENDIX**

Results and Discussion:

The total microbial viable count ranged from 1×10^5 to 3×10^5 CFU/g According to Food Safety and Standards Act, 2011 the maximum Viable count of Bacteria is 2,50,000 CFU /g and only 3 samples out of 20 shown the viable count more than that of the permissible range and the colonies of *Streptococci* and *Bacillus* were found.

Simple Staining: For the all the twenty samples crystal violet stain was used. Violet coloured, spherical shaped bacteria that are arranged in

singles, pairs, tetrads, short chains and irregular grape like clusters were seen which indicates the presence of cocci. Pink coloured, rod shaped bacteria are haphazardly present which shows the presence of bacilli

Indole test: Indole test is done for the detection of any presence of *Aeromonas hydrophilia*, *E.coli*, *Klebsiella oxytoca*, *Streptococcus faecalis* etc, 7 samples (presented in table 2) of 20 were positive to this Indole test

Voges prouskauer test: This test is used to detect the presence of microbes like *Enterobacter* species, Coliforms, *Klebsiella*, *Serratia marcescens*, *Vibrio alginolyticus* etc. If the result is positive it show a red colour solution will appear in 15-20 minutes of time or else it remains the same with no colour change. The following table showed the number of samples with positive result.

Catalase test: Among the twenty samples none of the sample showed any bubbling formation and hence doesn't contain or doesn't show the presence of *E.coli*, *Enterobacter*, and *Staphylococci* etc.

Citrate Utilisation test: Bacterial colonies are inoculated into slopes of Simmons citrate agar and incubated. The organism based on its ability to use citrate, changes its colour from green to blue. Among the twenty samples all the samples showed negative result.

Citrate utilization as a defining characteristic that could be used to distinguish between coli forms such as *Enterobacter aerogenes* which occur naturally in the soil, on plants, and in aquatic environments and faecal coliforms such as *Escherichia coli* whose presence would be indicative of faecal contamination.

Violet red bile agar test: The coli form group of bacteria includes aerobic and facultative anaerobic, gram-negative, non-spore forming bacilli. Coli forms ferment lactose and form acid and gas at 35°C within 48 hours. Members of *Enterobacteriaceae* comprise the majority of the group, but other lactose fermenting organisms may also be included.

For the respective twenty samples this test showed negative result and hence doesn't have any presence of coliforms in the samples.

Methylene blue reduction test: The methylene blue reduction test is based on the fact that the colour imparted to milk by the addition of a dye such as methylene blue will disappear more or less quickly. The removal of the oxygen from milk and

the formation of reducing substances during bacterial metabolism cause the colour to disappear.

Conclusion:

Overall, the results of the study indicate that the entire local made Cup packed ice cream samples studied in Hyderabad city are less contaminated. This may be attributed to the fact that ice cream mix has to undergo pasteurization process, and hence the microbial counts are expected to be low, less than 100 bacterial cells per ml [11,12]. The level of contamination of the packed ice cream as lower (10.7%) and may be this was a result of some of the organisms not surviving freezing temperatures for extended periods or being injured at these storage temperatures [13, 14]. Of these a majority (35%) of the samples analyzed was belonging to the family Enterobacteriaceae and these results are similar to the findings in Hyderabad city [15]. These are the organisms that are mainly found in the environment and some as part of the natural micro flora of humans [16]. These can usually get into ice cream from sources such as dust, soil, equipment and hands of handling personnel either during storage. 20% of the samples are found to contain coli forms. The presence of viable and possibly pathogenic bacteria such as *Escherichia coli*, *Klebsiella sp.*, *Staphylococcus sp* and *Bacillus sp.* in the analyzed Local brands of Ice cream samples should be viewed with concern by potential consumers, producing company and public authorities alike since food poisoning by these bacterial species is possible through consumption of contaminated ice cream. [17]. Hence necessary precautionary measures should be in place and has to be driven by the FSSAI authorities to prevent any such hazards by regular sample collection and Analysis and by creating awareness among the handlers the potential risk of hazards and the hygienic practices to be in place to prevent them.

SI – Sample Ice cream; +Ve: positive for Indole test

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APPENDIX MICROBIOLOGICAL REQUIREMENTS FOR MILK PRODUCTS (as per FSSAI)

(Ice Cream)

1. Total Plate Count	Not more than 2, 50,000/gm
2. Coli form Count	Not more than 100/gm
3. <i>E.Coli</i>	Absent in 1 gm
4. <i>Salmonella</i>	Absent in 25 gm.
5. <i>Shigella</i>	Absent in 25 gm.
6. <i>Staphylococcus aureus</i>	less than 10 per gm
7. Yeast and Mould count	less than 1 per g
8. Anaerobic Spore Count	Absent in 1 gm
9. <i>Listeria monocytogenes</i>	absent in 1 gm

Table – 1: Table showing the Total Viable count of 20 samples of Ice creams.

Samples	Trial 1 Cfu/g	Trial 2 Cfu/g	Trial 3 Cfu/g	Average Cfu/g	STDEV
SI 1	1.0 x 10 ⁵	2.0 x 10 ⁵	1.0 x 10 ⁵	1.3 x 10 ⁵	4.7 x 10 ⁵
SI 2	2.0 x 10 ⁵	1.4 x 10 ⁵	2.0 x 10 ⁵	1.8 x 10 ⁵	2.8 x 10 ⁵
SI 3	1.0 x 10 ⁵	1.5 x 10 ⁵	2.0 x 10 ⁵	1.5 x 10 ⁵	4.1 x 10 ⁵
SI 4	2.0 x 10 ⁵	1.2 x 10 ⁵	1.5 x 10 ⁵	1.5 x 10 ⁵	3.3 x 10 ⁵
SI 5	3.0 x 10 ⁵	2.0 x 10 ⁵	1.0 x 10 ⁵	2.0 x 10 ⁵	8.5 x 10 ⁵
SI 6	1.5 x 10 ⁵	1.6 x 10 ⁵	1.5 x 10 ⁵	1.5 x 10 ⁵	4.7 x 10 ⁵
SI 7	2.5 x 10 ⁵	1.8 x 10 ⁵	2.0 x 10 ⁵	2.1 x 10 ⁵	3.0 x 10 ⁵
SI 8	3.5 x 10 ⁵	2.5 x 10 ⁵	2.4 x 10 ⁵	2.8 x 10 ⁵	5.0 x 10 ⁵
SI 9	2.7 x 10 ⁵	2.5 x 10 ⁵	2.1 x 10 ⁵	2.4 x 10 ⁵	2.5 x 10 ⁵
SI 10	3.5 x 10 ⁵	2.8 x 10 ⁵	2.5 x 10 ⁵	2.9 x 10 ⁵	4.2 x 10 ⁴
SI 11	2.2 x 10 ⁵	2.5 x 10 ⁵	2.4 x 10 ⁵	2.4 x 10 ⁵	1.5 x 10 ⁵
SI 12	2.7 x 10 ⁵	3.2 x 10 ⁵	3.0 x 10 ⁵	3.0 x 10 ⁵	2.1 x 10 ⁵
SI 13	1.5 x 10 ⁵	1.6 x 10 ⁵	1.0 x 10 ⁵	1.4 x 10 ⁵	2.6 x 10 ⁵
SI 14	2.0 x 10 ⁵	2.5 x 10 ⁵	2.2 x 10 ⁵	2.2 x 10 ⁵	2.1 x 10 ⁵
SI 15	2.5 x 10 ⁵	1.8 x 10 ⁵	2.0 x 10 ⁵	2.1 x 10 ⁵	3.0 x 10 ⁵
SI 16	2.0 x 10 ⁵	2.4 x 10 ⁵	2.1 x 10 ⁵	2.2 x 10 ⁵	1.7 x 10 ⁵
SI 17	2.5 x 10 ⁵	2.1 x 10 ⁵	1.9 x 10 ⁵	2.3 x 10 ⁵	2.5 x 10 ⁵
SI 18	2.0 x 10 ⁵	1.8 x 10 ⁵	2.0 x 10 ⁵	2.0 x 10 ⁵	0.9 x 10 ⁵
SI 19	2.5 x 10 ⁵	2.0 x 10 ⁵	1.9 x 10 ⁵	2.0 x 10 ⁵	2.6 x 10 ⁵
SI 20	2.0 x 10 ⁵	1.4 x 10 ⁵	2.0 x 10 ⁵	1.8 x 10 ⁵	2.8 x 10 ⁵

SI: Sample Ice cream; CFU/g: Colony Forming Units per gram of sample

Table -2: Samples Showing Positive Results For Indole Test

SI 1	SI 3	SI 4	SI 8	SI 13	SI 15	SI 20
+ ve	+ve	+ve	+ve	+ve	+ve	+ve

SI – Sample Ice cream; +Ve: positive for Indole Test

Table - 3: Samples Showing Positive Results for Voges Prouskauer Test

SI 4	SI 5	SI 10	SI 14
+ve	+ve	+ve	+ve

SI – Sample Ice cream; +Ve: positive for Voges Prouskauer Test

Table - 4: Samples Showing Positive Results For Methylene Blue Reduction Test

SI 3	SI 4	SI 8	SI 11	SI 14
Reduced Colour	Reduced Colour	Reduced Colour	Reduced Colour	Little change

SI – Sample Ice cream;

Table- 5: Results of Biochemical Tests Performed on 20 Ice cream Samples:

SI; Sample Ice-cream; + Result is positive; - Result is negative; In: Indole Test; CU: Citrate utilization; VP test: Vogues Prouskauer test, VRBA test: Violet-Red Bile Agar test and MBR test: Methylene Blue Reduction test.

Samples	In Test	VP test	CU Test	VRBA test	Catalase Test	MBR test
SI 1	+	-	-	-	-	-
SI 2	-	-	-	-	-	-
SI 3	+	-	-	-	-	+
SI 4	+	+	-	-	-	+
SI 5	-	+	-	-	-	-
SI 6	-	-	-	-	-	-
SI 7	-	-	-	-	-	-
SI 8	+	+	-	-	-	+
SI 9	-	-	-	-	-	-
SI 10	-	-	-	-	-	-
SI 11	-	-	-	-	-	+
SI 12	-	-	-	-	-	-
SI 13	-	+	-	-	-	-
SI 14	-	-	-	-	-	-
SI 15	+	+	-	-	-	-
SI 16	-	-	-	-	-	-
SI 17	-	-	-	-	-	-
SI 18	-	-	-	-	-	-
SI 19	-	-	-	-	-	+
SI 20	+	-	-	-	-	-